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(73) Patentinhaber:

Socop Nahrungsmittel Handelsgesellschaft mbH, 1000 Berlin, DE

(74) Vertreter:

Ruschke, H., Dipl.-Ing., 8000 München; Ruschke, O., Dipl.-Ing., 1000 Berlin; Rotter, U., Dipl.-Chem. Dr.rer.nat., Pat.-Anwälte, 8000 München

(72) Erfinder:

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(56) Für die Beurteilung der Patentfähigkeit in Betracht gezogene Druckschriften:

NICHTS ERMITTELT

(54) Biophysikalisch derivatisiertes Hefepräparat, Verfahren zu dessen Herstellung und dessen Verwendung als Futtermittelzusatz

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VERIFIED TRANSLATION OF 15771/88

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Declaration

I, Ulrich Rotter, of Brunnerstraße 27, 8000 München 40, W.Germany,
(Translator's name) (Translator's Residence)

hereby declare:

1 am a citizen of the Federal Republic of Germany.

I am familiar with the German and English languages.

I have read International PCT application No. PCT/DE88/00212 filed March 31, 1988 and have understood the subject matter of said application.

I further declare that all statements made herein of my own knowlegde are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willingful false statement are punishable by fine and imprisonment, or both, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Munich, 17. 1o. 1989

U. Rotter
Signature

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Specification

The invention relates to a biophysically derivatized Ascomycetes or Schizomycetes cell material and in particular to a biophysically derivatized yeast preparation, having enhanced metabolic activity, a method of preparing the same, feed materials and plant growth compositions containing same and their use for skin treatment and for probiotic activation.

It has been known that plant and animal organisms have the ability to influence and activate the cellular metabolism. On this basis, numerous methods have been proposed for activating cell metaobolism. According to German Patent 1,076,888, respiration-promoting agents are recovered from dialyzeed blood cells or blood plasmas; they are supposed to increase the oxygen uptake of rat liver homogenate. Growth promoting products are obtained according to U.S. Patent 3,937,816 from the red pulpa of bovine spleen and, when injected intravenously, result in increased spleen weight in mice in a relatively short period of time. This prior patent includes no information on respiratory activity or influence on the phosphorylation of the respiratory chain.

It has also been known that therapeutically effective substances can be enriched in the yeast (cf. also DE-OS 33 41 840).

Algae, fungi and yeasts exhibiting enhanced metabolic activity such as to result in increased respiration of rat liver homogenate have not been known in the past. Conventional yeasts such as brewer's yeast (Saccharomyces cerevisiae and Saccharomyces carlsbergiensis), baker's yeasts (Saccharomyces)

Candida, Torula, etc., are inert relative to such homogenate. The respiratory enhancement factor is substantially equal to unity.

For the first time, the present invention provides metabolically active Ascomycetes or Schizomycetes cell materials and in particular a yeast preparation which exhibit respiration enhancing activity on standardized rat liver homogenate in the Warburg test. At 37°C, the respiration enhancing effect over a corresponding control sample generally is at least 2.o and preferrably has a value of 2.o to 1o.

The used expression "Ascomycetes" should include utricle fungi having utricular hyphae and forming ascospora, a member thereof form also the yeasts (Saccharomyces), and the yeast-like fungi the sporule formation thereof is still unknown, such as the genus Candida and Torulopsis. "Schizomycetes" are yeast-like bacteria. Within this description the term "fungi" will be used as an abbreviation for the groups mentioned above.

In the present description, the expression "yeast preparation" is used to denote whole yeast cells, yeast solution and yeast suspension, including yeast extract.

The respiration enhancement factor for the metabolically active yeast preparations of the invention is determined according to Warburg's method by its effect on rat liver homogenate and is the ratio of the additional oxygen (volume) respired by standardized rat liver homogenate relative to a control group of the same composition, but without the inventive preparation.

The increase of respiration and the respiration enhancement factor are measured in a Warburg vessel having a given volume and filled in accordance with a pre-established filling schedule with a buffer solution (preferably pH 7.4, Soerensen buffer), a specific amount of conditioned rat liver homogenate, and the substance or solution under test, with the substance under test (yeast) acting on the homogenate at 37° C for a period of 60 minutes. After the CO_2 formed by respiration has been absorbed quantitatively, O_2 consumption is determined by reading a pressure gauge. The oxygen consumption converted to normal conditions is divided by the consumption of a control sample without the substance under test. The quotient is a direct measure of the respiration enhancement factor.

The measurable increase in respiration caused by the inventive Ascomycetes, Schizomycetes and yeast preparations is dramatic, as is evident from the following Table I for a yeast product according to Example 1.

Table I

No.	Sample	Resp.Enh.Factor	Increase (%)
1	total solution	4.4	340
2	centrifuge supernatant	4.0	300
3	centrifuge residue	2.8	180
4	starting material	1.0	-

The characteristic feature of the inventive Ascomycetes, Schizomycetes and yeast products is a well-aimed modification of their biochemical behaviour, resulting in a substantial increase in metabolic activity. Criteria for an assessment of increased metabolic activity are - in addition to the previously discussed increase in respiratory activity of organ homogenates - improved wound healing, the positive effect on the reticuloendothelial system and growth-stimulating effects that have been found on fish as well as on warm-blooded animals. The metabolically active inventive Ascomycetes, Schizomycetes and yeast preparations show a positive effect also on the growth of normal yeast, as indicated by the following test results:

Table II

Yeast Cells

Yeast	Seed (cells/10 ml)	Harvest (cells/10 ml)	GEF(%)	GER(%)
a. Sabouraud maltose, 2% bouillion, normal yeast	2.0×10^6	1.0×10^9	500	-
normal yeast +2% inventive yeast product	2.0×10^6	1.5×10^9	750	50
b. Sabouraud maltose, 2% bouillion, normal yeast	5.0×10^6	2.0×10^8	40	-
normal yeast +2% inventive yeast product	5.0×10^6	2.6×10^8	52	30

(Table II cont'd:)
GEF = growth enhancement factor
GER = growth enhancement rate
Normal yeast (a) = Saccharomyces cerevisiae
Normal yeast (b) = Saccharomyces carlsbergiensis

Similar positive effects on growth were found also on bacteria such as species of Lactobacillus and Bifidus.

With respect to the changed biochemistry of the derivatized starting products based on Ascomycetes, Schizomycetes and yeast cells it is interesting to examine the TLC diagrams of lipids, amino acids and nucleinic acid components obtained under definite test conditions and compared to the starting cells which are employed for derivatization.

The twodimensional TLC diagrams obtained are useful for characterization of the derivatized products and for distinguishing them against products obtained by differ ent methods.

Fig. 1 shows a twodimensional lipid TLC diagram of the derivatized yeast. 1 g of derivatized yeast was extracted with 4 ml $CHCl_3/CH_3OH$ (1:3). The extract was analysed by chromatography: 1. eluent $CHCl_3:CH_3OH:H_2O$ (6o:3o:5), 2.eluent n-butanol: $CH_3COOH:H_2O$ (4:1:1).
To detect the lipids the plate was sprayed with Primuline reagent and evaluated by means of an UV lamp at wavelength of 366 nm.

Surprisingly, most of the inventive Ascomycetes, Schizomycetes and yeasts were found to have - in addition to respiration enhancing activity an inhibiting factor acting on oxidases, of which amino acid oxidase [1.4.3.2. and 3.], monoaminooxidase [1.4.3.4.] and diaminooxidase [1.4.3.6.] should be mentioned explicitly. Caused by this effect, the oxidation of L-amino acids (which actually is catalyzed by L-amino acid oxidase [1.4.3.2]) presumably is inhibited or retarded. L-amino acid oxidase is present in many nutrient consuming systems, such as the intestinal flora and soil cultures. A direct effect of this inhibiting factor is a higher percentage of L-amino acid utilization from the nutrients supplied to such a system, the result being that the animal or plant can grow more rapidly.

It has not yet been established safely at this time that this inhibiting factor is related to - or at least correlates with - the enhanced respiratory activity.

Without desiring a commitment to any specific mechanism, one could explain the effect of this inhibiting factor as follows: L-amino acid oxidase contains FMN (flavine

mononucleotide) firmly bonded as a prosthetic group. As L-amino acids are oxidized, their amino group is oxidized according to the following mechanism:

1. L-AA + H_2O + enzyme-FMN
$$\longrightarrow \text{alpha-keto acid} + NH_3 + \text{enzyme-FMN.}H_2$$

2. Enzyme-FMN.H_2 + O_2 --> enzyme-FMN + H_2O_2

The oxygen consumption can be measured in consideration of the amount of H_2O_2 formed as H_2O_2 decomposes to H_2O and 1/2 O_2. In the presence of catalase, this decomposition is spontaneous and quantitative so that the total reaction of the L-amino acid oxidation corresponds to equation 3:

3. $$\underset{\substack{|\\ NH_2}}{R-CH}-COOH + 1/2\ O_2 \longrightarrow R-CO-COOH + NH_3$$

If in a system L-amino acid oxidase is inhibited or blocked or immobilized in any other way, less oxygen will be consumed than in the absence of the inhibiting factor so that amino acids and proteins will be available in greater amounts, potentially resulting in enhanced and more intensive utilization of nutrients.

For determining the L-amino acid oxidase inhibiting factor (IF) of the inventive Ascomycetes, Schizomycetes and yeasts, L-amino acid oxidase solution (1 mg/ml), catalase suspension, pH 7.8 buffer solution (5o ml o.2M $Na_2P_2O_7$ + 37.5 ml 0.2M HCl), L-phenylalanine solution (1 mmol/50 ml, buffer pH 7.8) and test solution (centrifugate, 10^3g, or yeast suspension) are filled into a Warburg vessel in accordance with a

pre-determined filling schedule so that the measurements include the reaction of L-amino acid oxidase and L-phenylalanine as well as the reaction of the enzyme and the test solution (without phenylalanine), and of the enzyme with the test solution plus phenylalanine. The cylinders are provided with filter paper strips impregnated with 6N KOH. The solutions are equilibrated at 37° C; the enzyme solution is then filled into the main chamber containing the test solution or a blind solution so as to start the reaction. The microliters of oxygen consumed can then by recorded as a time function; it constitutes a direct measure of the inhibiting factor if correlated with the oxygen consumption of the blind test. In general the reading for determining the IF is taken after 60 minutes.

For evaluation, a sum curve is formed of the test curves "L-amino acid oxidase + phenylalanine" and "L-aminoacid oxidase + yeast solution" (oxygen demand is caused by the amino acids contained in the yeast itself) and the sum curve (a measure of the oxygen uptake to be expected theoretically) is related to the curve "L-aminoacid oxidase + L-phenyl-alanine + yeast solution". In the case of the bio-physically derivatized inventive Ascomycetes, Schizomycetes and yeast preparations, the resultant re-duced oxygen consumption expresses the percentage inhibit-ing factor (such as o.42 = 42% inhibition , i.e. reduced oxygen consumption). Compared with the respective starting cells , which correspond practically to the theoretic sum curves, the IF is impressive and may reach levels as high as 70 % or even 80 %.

Another surprising property of most of the inventive Ascomycetes, Schizomycetes and yeast preparations is their wound healing activity, which may be evaluated by

observing the healing of dorsal cuts on rats. After six days, wound tension on wound of the animals so treated was markedly better than in a control group. The advance of the aninmals so treated over control was measurable as late as the 21st day.

In these tests, male Wistar rats having a body weight of 210 - 230 grams were anesthesized and their dorsal hair was shaved. Then, a linear cut of about 4 cm length was applied along the medial line and then sutured at three locations spaced one centimeter. The wound was opened on the 6th, 11th, 16th and 21st day after the operation and three narrow slices of skin were taken at right angles to the wound line, including the 1 cm width of the wound line itself. Thereafter, the tension caused by withdrawing the skin portions was measured.

2.5 ml/kg of the centrifugated test solution supernatant from example 1 were administered on the day of the intervention and then each day. The control group was fed 2.5 ml/kg of physiological saline (NaCl) solutions, all other condition being the same. In each test, 25 animals were tested per group.

In the observations, the tension of the wounded skin portions increased steadily on the animals treated with the test solution. In each daily test, the treated animals showed a higher skin tension than the control group. In the initial condition on the sixth day, the difference to the control group was higher than 100%. The results are listed in Table III.

Table III

No. of Days	Test Group *)	Control
6	215±17	104±33
11	493±24	390±23
16	840±48	730±30
21	1659±52	1401±60

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*) The test group was given 2.5 ml test solution per kilogram of body weight.

One explanation of the markedly improved healing of wounds is that the inventive yeast promotes tissue respiration and intensifies metabolic functions so that tissues regenerate much more rapidly.

The inventive preparation, for instance the derivatized yeast, has a beneficial influence not only on wounded skin, but also on unwounded sound skin. With a biophysically derivatized yeast preparation (ex. 1) the moisture maintainance capability of human skin was normalized and often improved. In order to demonstrate this effect the water content of skin was determined before and after treatment for soem hours. Compositions containing the preparation were applied to dry skin, i.e. on skin having poor moisture balance. On same skin a control sample having same composition but without the preparation was also applied. It resulted that the water content was enhanced and the moisture maintainance capability practically was doubled. Fig. 2 shows the curves of treated skin and untreated skin. On the X axis hours after treatment and on the Y axis moisture in percent are graphed.

As already explained in connection with wound healing, skin elasticity and skin tension will be improved by compositions containing the inventive fungi or yeast preparation. This is well established by resonance frequency measurements conducted on human skin.

Another criterion for the enhanced metabolic activity of the inventive Ascomycetes, Schizomycetes and yeast preparations is their positive effect on the reticuloendothelian system. An RES activating effect can be stated for the novel inventive preparations on the basis of their effect on RES function in rats - inhibited by tryphan blue - according to the Kongorot method. To this end, 1 ml/kg of a 1% Kongorot solution was injected intravenously into male Wistar rats of 210 - 230 g body weight; blood samples were taken after 4 and 60 minutes. The Kongorot coefficient followed from the decadic extinction ratio at 500 nm of the serum diluted by 10. For inhibiting RES system functions, 10 ml/kg of 1%

tryphan blue solution were administered intraperitoneally. Inhibition was calculated as corrected decadic extinction at 500 nm from a measurement of decadic extinction at 650 nm. The tryphan blue solution was administered 6.5 hours before RES system examination using the Kongorot method. In the test, o.5 ml of the test solution (centrifuge supernatant according to Example 1) were administered intraperitoneally 5 hours before the examination

The control group was given an adequate amount of physiological NaCl solution. A total of 25 animals were tested per group.

The average Kongorot coefficients were found to be 9.4 for the treated group and 16.1 for the control group. This result can be considered an direct proof of the enhancement of RES system functions by the inventive yeasts as the inhibiting effect of tryphan blue has been cancelled.

The intensification of metabolic activities the inventive Ascomycetes, Schizomycetes and yeast preparations produce is quite surprising since the Ascomycetes, Schizomycetes and yeast cells as well as their cell preparations are treated by physical methods exclusively. In the present application, this treatment is referred to generally as "derivatization". The Ascomycetes, Schizomycetes and yeast cells can be derivatized by low temperature treatment, laser irradiation, insonification and other biophysical procedures as well as by a relatively short and intensive heat treatment. The treatment obviously results in a marked reduction of cell size, preferrably to one half or less, of the original diameter, while cell wall thickness increases so that the average cell diameter to cell wall thickness ratio changes by a factor preferrably is in the range of 5 to 20.

Preferrably the preparation of Ascomycetes and Schizomycetes cell materials additionally comprises a small amount of mitochondriae extract whereby the growth promoting activation of the preparation is additionally enhanced. The mitochondriae extract is conveniently added already before or during the biophysical treatment. Mitochondriae (chondriosomes) are formvariable inclusions within the protoplasma of single cells and of plant and animal cells, having a width of about o.1-1 μm and a length of 1-5 μm. It is commercially available as pyrogenfree dry powder or as solution, such as the Mitochondyl powder of Widmer AG, Swizzerland, which has about 2 % nitrogen and about 5o % dry substance content. This product of commerce is utilized in the examples.
In general the mitochondriae extracts are prepared by cell cleavage, frequently yeast cells, and subsequently suitable steps of rehydrating, suspending, washing and drying, see P.M. Nossal, Austral. J. exp. Biol. med. Sci. 31, p. 583 (1965); M. Merkenschläger, K. Schloßmann, W. Kurz, Biochem. Z. 329, p. 332 (1957). Mitochondyl is a product comprising extracted yeast cell mitochondriae mixed with a buffer substance. It is added directly as white powder or after preparing a buffered solution which also contains preservation agents. It is preferred to use an amount of mitochondria extract of about o.o5 to o.5o wt.%, calculated as dry substance and related to the dry solid content of the cells to be derivatized.

Under the given conditions, during the treatment the Ascomycetes, Schizomycetes and yeast cells practially do not multiply any more; neither is such multiplication desired.

The preferred vehicle consists of

(a) a small amount (preferably 0.2 - 5.0 wt.%) of bio physically derivatized yeast and/or chondriosome extract (preferably 0.01 - 0.1 wt.%) recovered from the yeast used as starting material or from any other yeast source;

(b) cellulose glycolates, pyrogenic silica, tragacanth and/or carrageens;

(c) two fermentable mono- and/or disaccharides;

(d) preserving agents; and

(e) trace elements such as Mn, Co, Zn, Mg in the form of sulfates or gluconates.

Trigger yeast (a) is used to start the treatment, additive (b) ensures improved yeast distribution. The carbohydrates of (c) prevent fermentation of the cell inherent carbohydrates. The preserving agents of (d) preferably are parabene (4-hydroxybenzoic alkyl esters), sodium hydroxide and calcium chloride to protect the end product against the microorganisms from the start. The trace elements of (e) are added in order to adjust the desired concentrations thereof in the yeast cell or the yeast cell suspensions.

In addition, the inventive Ascomycetes, Schizomycetes and yeast products positively influence the growth of microorganisms such as Bifidus, Lactobacillus, Cocci, soil bacteria and intestinal bacteria.

In higher plants, the biophysically derivatized Ascomycetes, Schizomycetes and yeast preparations of the present invention have a marked effect on plant growth, inflorescence and fructification. With up

Ascomycetes, Schizomycetes and yeast to 1% of the liquid preparations according to the following examples 1 - 6 added to the watering water, ornamental plants such as tagetes, petunia, lobelia, chrysanthemum and carnation grow at least twice as fast than a control sample in a 6 week test period and develop abundant blossoms

and leaves. Similar improvements have been observed in useful plants such as peas, sunflower and corn; they demonstrate that the inventive preparations are suited very well for use as growth stimulating additives to watering and irrigation systems. This favorable effect may be explained by the positive effect the inventive preparations produce on the microflora of soils.

Example 1

30 parts by weight of 95% dry yeast (Saccharomyces cerevisiae) and 70 parts by weight of an aqueous solution or suspension of a vehicle containing (by weight) 0.5% of biophysically derivatized yeast, 0.03% chondriosome extract, 0.6% cellulose glycolates, 0.1% parabene, 2% salt, 0.1% total of glucose and saccharose, 0.00001% trace elements are irradiated at 15 - 25°C with a conventional CO_2 laser. A laser power output on the order of 0.3 watts is totally sufficient; focussing is to 0.05 - 5 kW/cm².

The device used for containing the suspension for irradiation by the CO_2 laser comprises two suitably sized vessels connected at the bottom by a tube of infrared glass having a length of 20 - 50 cm and an inner diameter of 3 - 6 mm. 20 liters of the suspension are pressed with the aid of

ultra-pure nitrogen into the nitrogen-purged left vessel of the assembly. After the laser has been adjusted by means of an endoscope to a spot size corresponding to the inner diameter of the connecting tube, the suspension is pressed with the aid of nitrogen from one vessel into the other, with the second vessel also being filled with nitrogen. Using 0.3 W power and a suspension flow rate of 20 to 200 ml/min, the treatment is performed until the desired cell reduction has been obtained, as may be verified by means of a microscope. Thereafter, the suspension is heated shortly (not more than 25 min) to more than 50 C and is held at the end temperature while stirred. Cooled to 30°C, the suspension is preserved by adding 0.1 - 0.2 wt.% of a preserving agent such as sorbic acid and/or nipagin.

Morphologic cell examination showed a reduction of the average cell diameter to about 50% and a marked increase of cell wall thickness. Both the total product (suspension) and the fractions obtainable by centrifugation (10,000 rpm), i.e. supernatant and residue, show metabolic activity in the Warburg test (in contrast to the starting yeast) when tested as described above for the respiration enhancing effect on rat liver homogenate. The respiration enhancement factor for the total product and for the centrifuge supernatant was higher than 4 (cf. Table I). Further activities were shown by the wound healing test (Table III) and in the RES activation test described above.

The total product was tested in dosages of 2 permill added to the preparatory feed of piglets and weaned pigs of 10 - 35 kg weight (cf. Tables IV and V, respectively). Improvements in daily feed intake were about 58% and 42%,

respectively and clearly higher than with commercial feed additives, resulting in feed utilizations improved by 16.7% and 19.5%, respectively. Details and results are listed in the following Tables IV and V.

Table IV

10 - 35 kg Live Weight Piglets

	Control (w/o)	Com.Preparations A	Com.Preparations B	Test Prepar. (2 o/oo)
Specimen/group	24	24	24	24
Init.weight (kg)	10.2	10.1	10.3	10.2
Test period (days)	35	35	35	35
Final weight at end of test (kg)	25.4	26.7	30.2	34.2
wt.increase (kg)	15.2	16.6	19.9	24.0
daily increase (g)	434	474	569	686
difference (%)	100	109	131	158
feed intake (g) p.day & animal	908	971	1093	1194
feed(kg) per kg wt.increase	2.09	2.05	1.92	1.74
difference(%)	100	98	92	83.3
Improvement:				
daily increase (%)		9.21	30.9	57.9
feed utilization(%)		2	8	16.7

(Table IV cont'd:)
Commercial preparations: A = CTC; B = bayo-nox
(as in Table V).

Table V

5 - 12.5 kg Live Weight Pigs
Weaned After 21 Days

	Control (w/o)	Comm.Preparations A	Comm.Preparations B	Test Prep. (2 o/oo)
Specimen/group	24	24	24	24
Init.Weight (kg)	4.93	5.14	5.12	5.06
Test Period (days)	20	20	20	20
Weight at end of test (kg)	10.2	11	11.9	12.9
Total increase (kg)	5.23	5.84	6.78	7.45
Daily increase (g)	262	292	339	373
Difference (%)	100	112	130	142
Feed intake (g) p.animal & day	429	465	480	492
Feed (kg) p.kg of weight increase	1.64	1.59	1.42	1.32
Difference (%)	100	97	86.3	80.5
Improvement:				
daily increase (%)		11.7	29.6	42.2
feed utilization (%)		3	13.7	19.5

Example 2

100 liters of a suspension of Torulopsis utilis and Saccharomyces cerevisiae (2:8 mix) with 20% total dry solids were mixed with a composition containing:

1	kg	product from Example 1
0.05	kg	chondriosome extract
0.1	kg	cellulose glycolate
0.1	kg	gum arab
0.1	kg	glucose + mannose
0.1	kg	parabene
1.5	kg	of a salt mixture consisting of NaCl, $MnSO_4$ and $MgSO_4$ and
0.00001	%	trace elements.

The suspension is stirred and ultrainsonified at 20° C and during insonification is heated initially to 45° C and then shortly (2 min) up to 80° C.

The cooled suspension is spray-dried with 2oo g silica (Aerosol 300) and a sufficient amount of nipagin (preserving agent) in suitable apparatus (e.g. Büchi device), yielding about 1.5 kg of solid product per hour.

Examination for respiration increasing activity on rat liver homogenate resulted in a respiration enhancement

factor of 5.1 (liquid product, prior to spray-drying). The wound healing test produced a 100 % improvement over control (measurement of skin tension as described). In contrast, the starting yeast was inactive.

The intensified metabolic activity the novel preparations produce was demonstrated also in growth tests on fish and

chicken. Even very small amounts added to normal nutrition (less than 1 o/oo) resulted in a surprisingly pronounced effect on growth, as manifested by substantial feed savings. Tables VI and VII summarize these results.

Table VI

Growth Tests on Sword Fish

	Control	0.8 o/oo add.	0.95 o/oo add.
Aver.weight of young fish aft. 47 days	1.9	3.7	4.1

In the test, 60 young fish 15 days old were divided into the three groups and, held in 5 liters of water at 24.5 C average water temperature, were fed twice each week. Two groups of fish were given 0.8 and 0.95 /oo, respectively, of the additive (derivatized yeast) from example 2, distributed among three feedings per week.

The weight increase is extraordinary and shows the growth promoting effect of the inventive preparation.

The spray-dried product from Example 2 was tested as an additive to the feed of Harvard chicken. A test group of 3,600 chicken was fed with 0.1 wt.% of the additive added to their feed for 60 days. A control group of 3,600 chickens received the same feed without the additive. In addition to mortalities, body weight was determined on groups of 20 chicken (Table VII).

Table VII

Chicken Feeding Test

Day No.	1	10	20	30	40	50	60
Weight (g) of test grp.	34	209	568	1110	1590	2001	2090
Weight (g) of control	39	246	550	1100	1510	1890	1990

The resultant feed intake (kg) per kilogram of body weight was 2.27 for the test group and 2.65 for control; mortalities were 4.9% for the test group and 7.7% for control. The improved feed utilization corresponds to feed savings of about 15 %.

Example 3

10 kg of compressed yeast (Saccharomyces cerevisiae) are cooled in suitable refrigeration means to a temperature below -13° C and conditioned at that low temperature for about 4 hrs. Thereafter, there are added to the product 500 ml of an aqueous suspension containing 15 g fructose, and saccharose, 40 g finely particulate silica (SiO_2), 50 g of the preparation from example 2 (based on the solid product), 15 g parabene and 160 g NaCl; initially, the mixture is mixed intimately and heated to room temperature (about 23°C), causing a liquid mixture to form which readily suspends the yeast particles. The mixture is rapidly heated at a rate of about 5 degrees temperature increase over each 3 min. period to a temperature between 45°C and 75°C while stirring constantly.

The resultant low-viscous yeast suspension of readily re-

suspended particles has in its entirety, but also as a centrifugate (supernatant or residue), a respiration enhancement factor of 4.8.

Example 4

10 kg of compressed yeast (Saccharomyces cerevisiae) are cooled to a temperature below -15 C. After 24 hrs. there are added: 10 g parabene, 5 g sorbic acid, 170 g NaCl as preserving agent and 10 g saccharose, 15 g SiO_2, 10 g of cellulose glycolate and 0.8 mg trace elements (Mn, Co, Zn, Mg).

Having reached room temperature (25° C),the mixture is fed under stirring into the Laser apparatus described in Example 1 and treated with the laser radiation until the the yeast particles have shrunk to 50% and less of their diameter prior to the laser treatment. Thereafter, and under continued stirring, the product is heated for a short time (max. 20 min.) to a temperature between 40° C and 80° C.

The respiration enhancing factor of this product is 5.1. Following preservation, the suspension is spray-dried. The product can be used as start yeast for the biophysically derivatized product in Examples 1 - 3. It was used for feeding experiments, with the metabolism increasing activitiy being comparable to the results of Example 2 when tested as a feed additive for Harvard chickens. In practical applications, this activity amounts to substantial savings in feed materials and strongly reduced feeding periods, a feature of particular merit for large-scale tests.

The novel preparation showed particular utility in the feeding of fur animals such as chinchilla. The lanugo hair becomes very smooth and shiny a short time after initial administration of the preparation. The fur of older breeding animals continues to be usable if they have been fed the inventive yeast preparation for some time.

Example 5

5oo kg of 2o% yeast (Saccharomyces), so-called yeast-2o, at 2o^{o}C were added with o.2 kg of parabene, o.o3 kg of sorbic acid, 2 kg of sodium chloride, o.2 kg of saccharose, o.5 kg of SiO_2, o.3 kg of cellulose glycolate, o.5 kg of mitochondria extract and 1o mg of trace elements and stirred with a Ultraturax so as to cause rap-id heating of the mixture. At 55^{o}-6o^{o}C stirring was continued for about 1o minutes and subsequently it was cooled. Feed tests succeeded in comparable results as achieved in ex.s 1 and 2.

Example 6

5o kg of yeast-2o (Saccharomyces) at 2o^{o}C were added with 2o g of glucose plus fructose (1o:1), 4o g of alginate, 3o g of mitochondria extract, 18o g of sodium chloride, 1o g of sodium ascorbate as preserving agent and 1.5 mg of trace elements and stirred with a Ultraturax while insonificated at same time. The mixture temperature raised to 55^{o}C without external heat supply. It was held at 52-6o^{o}C for short time and no longer than 2o minutes. The cooled mixture was utilized for enhancing the yoghurt production by adding less than o.1 wt.% thereof to the culture medium, related to the total weight of the medium. The product of this example was also useful for other purposes of diary product industry since the growth of microflora was accelerated, respectively.

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SOCOP Nahrungsmittel GmbH,
Wallenroder Straße 6, D-1ooo Berlin 26

Biophysically Derivatized Ascomycetes, Schizomycetes and Yeast Preparation, Method of Preparing Same, and Feed and Plant Growth Compositions Containing Said Preparation and Use of the Preparation for Skin Treatment and Probiotic Activation

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Patent Claims

1. Preparation comprising biophysically derivatized Ascomycetes or Schizomycetes cell material, having respiration-enhancing activity measurable in a Warburg vessel with standardized rat liver homogenate and expressed as the oxygen volume additionally respired by the homogenate.

2. Biophysically derivatized yeast preparation having respiration-enhancing activity measurable in a Warburg vessel with standardized rat liver homogenate and expressed as the oxygen volume additionally respired by the homogenate.

3. Preparation as in claim 1 or 2, characterized in that the respiration increase factor at 37°C is at least 2.o, relative to the homogenate under otherwise equal test conditions.

4. Preparation as in claim 1 or 2, characterized in that the respiration increase factor at 37°C has a value of 2.o to 1o and preferrably a value of 2.5 to 5.5.

5. Preparation as in claim 1 or 2, characterized by additionally including an L-amino acid oxidase inhibiting factor measurable in the Warburg test with a standardized test solution with the enzyme plus phenyl alanine as the L-amino acid and expressed as the ratio of oxygen consumption with and without said preparation.

6. Preparation as in claim 5, characterized by the L-amino acid oxidase inhibiting factor having a value in the range of about 2o to 8o % and preferrably a value in the range of 3o to 6o %.

7. Preparation as in claim 1 or 2, characterized by having wound healing activity.

8. Preparation as in claim 1 or 2, characterized by additionally exhibiting RES activating activity.

9. Preparation as in claim 1 or 2, characterized by positively influencing the growth of yeasts and microorganisms such as Bifidus, Lactobacillus, Cocci and soil bacteria, whereby it indirectly promotes the growth of higher plants and algae.

1o. Preparation as in claim 1 or 2, characterized by additionally containing mitochondriae extracted from animal and/or plant cells.

11. A method of preparing a preparation comprising biophysically derivatized Ascomycetes or Schizomycetes cell material, having respiration-enhancing activity, characterized by treating Ascomycetes or Schizomycetes cells - optionally

with a liquid vehicle added- by laser radiation, freeze drying, ultrainsonification, short-time pressurization and/or osmolysis for a time sufficient to convert the cells to a readily re-suspendable material, and by subjecting the suspension of contracted cells to a short-time intensive heat shock treatment until gases formed by fermentation begin to escape.

12. A method of preparing a biophysically derivatized yeast preparation having respiration-enhancing activity, characterized by treating a starting yeast -optionally with a liquid vehicle added- by laser radiation, freeze drying, ultrainsonification, short-time pressurization and/or osmolysis for a time sufficient to convert the cells to a readily re-suspendable material, and by subjecting the suspension of contracted cells to a short-time intensive heat shock treatment until gases formed by fermentation begin to escape.

13. A method as in claim 11 or 12, characterized by the heat treatment leaving the cells at a temperature above 4o°C for a period not longer than 25 minutes.

14. A method as in claim 11 or 12, characterized by said heat treatment being effected by means of hot gas supplied in counter-current.

15. A method as in any one of claims 11 to 14, characterized by the biophysical cell reduction being performed at the same time as the short-time intensive heat treatment.

16. A method as in any one of claims 11 to 15, characterized by adding for the liquid vehicle an aqueous solution or suspension containing:

(1) o.1 - o.5 % biophysically derivatized yeast obtained from a yeast serving as starting material and/or another yeast source, and o.o2 - o.4 % chondriosome extract;
(2) o.3 - 5 % cellulose glycolate, highly dispersed pyrogenic silica having more than 99,8 % SiO_2 and a diameter of 7 to 25 nm, carrageen, tragacanth or other similar fillers;
(3) o.o1 - o.3 % fermentable mono- and/or disaccharides;
(4) preserving agents such as parabene and salts such as NaCl in sufficient concentration; and
(5) trace elements such as Mn, Co, Zn, Mg preferrably in the form of sulfates or gluconates.

17. A method as in any one of claims 11 to 16, characterized by using for the starting cell material a strain from the Saccharomycetaceae family.

18. A method as in any one of claims 11 to 17, characterized by treating a dry yeast having a 95 % to 98 % HTS content together with two to four times the amount of a liquid vehicle.

19. Feed material, characterized by comprising a conventional feed having added thereto up to 1 wt.% of the biophysically derivatized preparation having enhanced respiration activity as claimed in any one of claims 1 to 18.

2o. Plant growth promoting solution comprising watering water and up to 2 wt.% of the biophysically derivatized preparation as claimed in any one of claims 1 to 18.

21. Use of the biophysically derivatized preparation as claimed in any one of claims 1 to 1o for skin treatment or skin conditioning.

22. Use as in claim 21 for normalization and increase of the skin moistening capability.

23. Use as in claim 21 for increasing the skin elasticity.

24. Use as in claim 21 for skin wound healing.

25. Use as in claim 21, in combination with a known skin healing and/or skin conditioning agent such as collagen, organ extracts, plant extracts or polyvalent alcohols having 2 to 8 carbon atoms.

26. Use of the biophysically derivatized preparation as claimed in any one of claims 1 to 1o for probiotic activation of dairy products, whereby the derivatized cell material is effecting growth stimulation together with selected natural microorganisms.

27. Use as in claim 26 in the production of yoghurt.

28. Use as in claim 26 for growth stimulation of Lactobacillus bulgaricus.

29. Use of the biophysically derivatized preparation as claimed in any one of claims 1 to 1o for probiotic activation

of Actinomycetes and in particular for activation of yeasts.

3o. Use as in claim 29 for activation of yeasts selected from the group consisting of Aspergillus niger, Torula utilis, Neurospora crassa, Saccharomyces strains and Japanese ikashio-kara yeast.

Abstract of the Disclosure

Ascomycetes, Schizomycetes or
A biophysically derivatized yeast preparation exhibits respiration enhancing activity on liver homogenate, includes an L-amino acid oxidase inhibiting factor and shows pronounced RES-activating and wound healing effects. The method of preparing this yeast product provides for the treatment of a starting yeast - optionally with a vehicle added thereto - until a readily re-suspended mass is obtained, which is heat treated for a short time until gases formed by fermentation start to escape. The invention also proposes feed materials and plant growth solutions including the biophysically derivatized yeasts as additives
Ascomycetes, Schizomycetes and
The preparations are also useful for skin treatment and for probiotic activation of diary products.

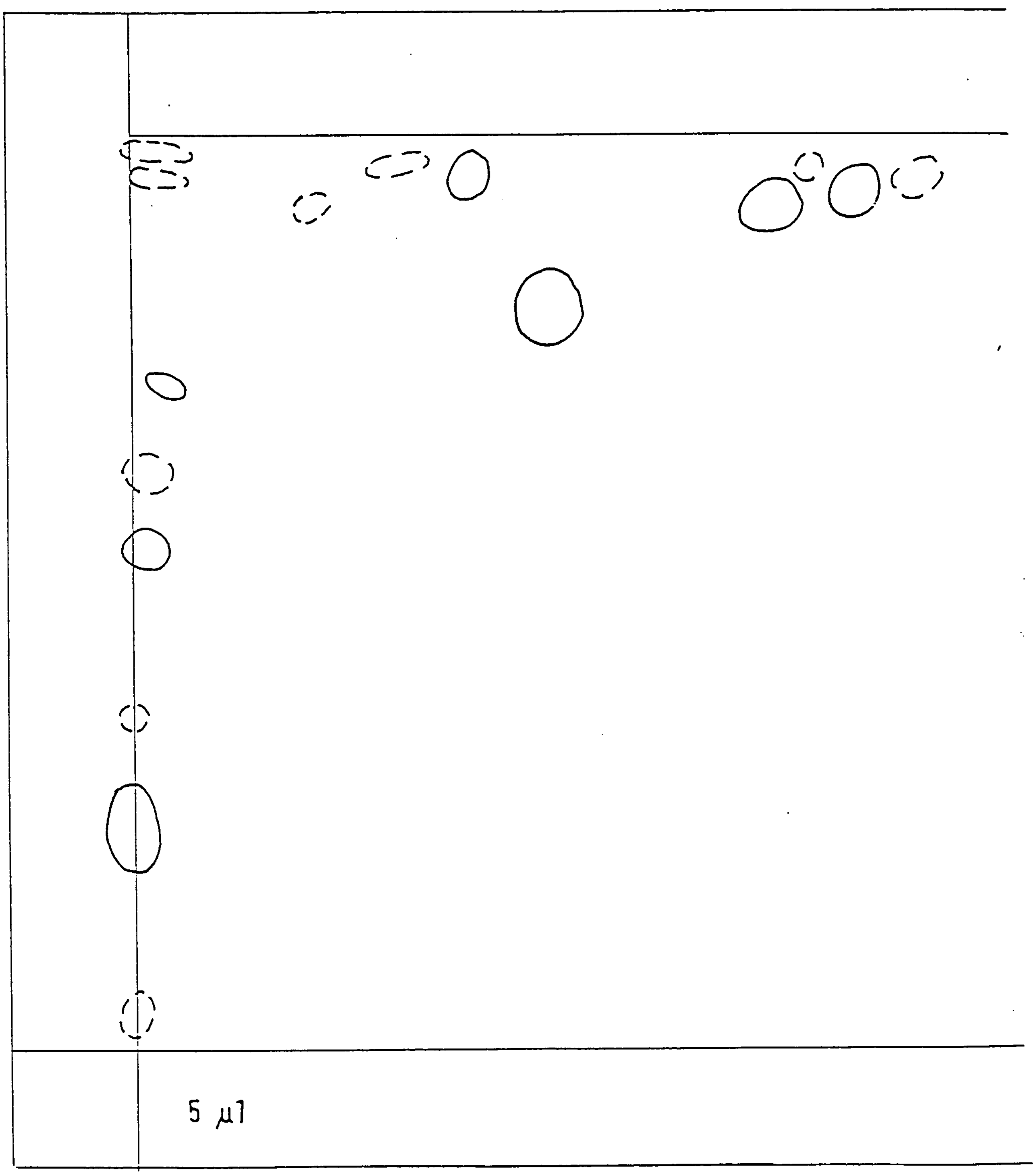

FIG. 1

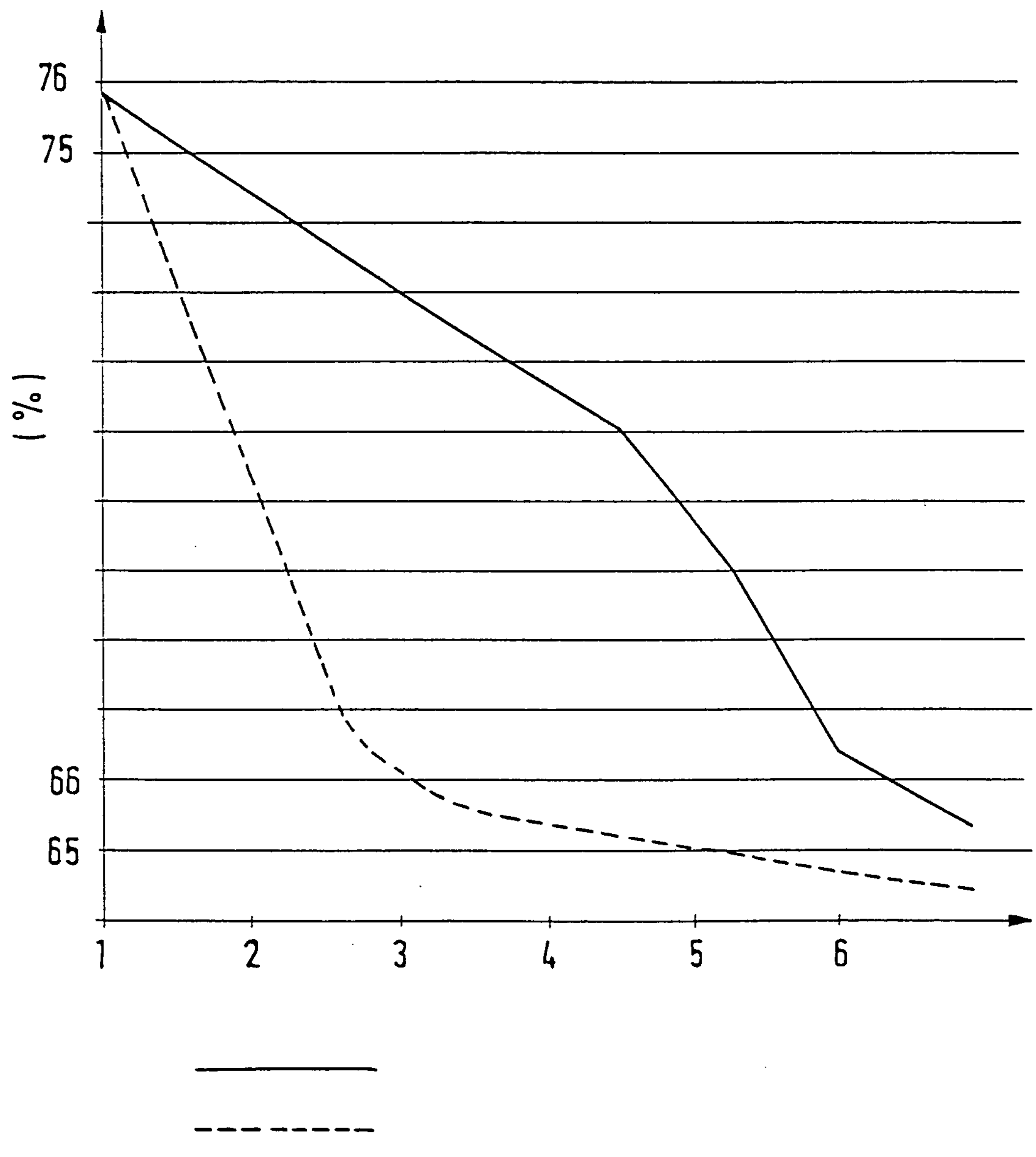

FIG. 2